REMARKS

Objection to FIG. 3B

In response to the objection to previously filed drawings for FIG. 3B, as allegedly being "jumbled," it is pointed out that the drawings submitted September 20, 2005 for FIG. 3B are in fact fully proper in form and in full compliance with the requirements of 37 CFR §1.84.

Specifically, applicant increased the font size in the FIG. 3B drawings filed September 20, 2005 as had previously been requested by the examiner. These revised FIG. 3B drawings were generated from the same program file within the applicable software program ("Gene Construction Kit"), and the only change was that the font size of characters in the drawing sheets for FIG. 3B was increased.

Thus, the FIG. 3B drawings filed September 20, 2005 are identical to the drawings of FIG. 3B originally filed in the application, the only change being that the font size has been increased, as a result of which the number of pages is increased (to six sheets). Due to the increased font size, the numbering that depicts the very first nucleotide (or amino acid) at the very beginning of each lane has correspondingly been shifted. This shifted numbering is in fact unavoidable when, as requested by the examiner, the font size of the characters is changed.

The examiner therefore is requested to reconsider the objection to the drawings on the basis that the September 20, 2005 drawing sheets for FIG. 3B are in fact fully in proper form and compliance with 37 CFR 1.84.

Objection to the Specification and Rejection of Claims and Traversal Thereof

In the February 16, 2006 Office Action, the specification was objected to and claims 1-14 and 21-24 were rejected under 35 U.S.C. §112, first paragraph for reasons of record.

These rejections are hereby traversed and reconsideration of the patentability of the pending claims is requested in light of the following remarks.

Sufficiency of Written Description and Patentability of Claims 1-14 and 21-24

Applicants concur with the examiner's statement that "[h]ybridomas survive in the HAT selection medium, period" (page 3, lines 17-18 of the February 16, 2006 Office Action).

Applicants also concur with the examiner's statement that "[w]hat other properties are retained by such fused cells would seem to be entirely unpredictable because of the ... random shedding of genetic elements from hybridomas and thus, the need to select relevant cells secreting relevant antibodies from the population of fused cells" (page 3, lines 18-21 of the February 16, 2006 Office Action).

This last statement, correctly advanced by the Examiner, leads to the crux of the invention: the generation of a physical linkage of a produced antibody to the very cell that encodes this specific antibody. Surface displayed antibodies thereby enable a selection of the antibody-producing cell. Contrary to conventional hybridoma technology, this surface display of antibodies obviates the time-consuming and labor-intensive necessity to separate individual clones, e.g. in wells, and instead enables the selection of antibodies from pooled hybridoma cells in a simple and efficient manner by use of state of the art technology, e.g., by FACS sorting.

The Office has contended that "one skilled in the art ... would not be assured of the ability to select the desired producer cell(s) from the population because, absent further guidance from applicant, one would be unable to identify and specifically separate the secreting cell(s) from a population of cells which are capable of binding the secreted product." Applicants disagree with such statement.

The Office bases its claim that there is insufficient disclosure of the invention on the CAFC's pronouncement in *Genentech Inc. v. Novo Nordisk* that "when there is no disclosure of any specific starting material or of any of the conditions under which a process is to be carried out, undue experimentation is required."

Applicants in response point to the fact that to practice applicants' invention the only specific starting material needed to generate hybridoma cells with surface displayed antibodies is a myeloma cell line that is engineered to display antibody binding proteins, e.g., protein G linked to the membrane.

Such a cell line, and the DNA used to generate it, are disclosed in the present application, such cell line is available, such cell line exhibits a stable display of large amounts of protein G over a period of months, and such cell line furthermore was tested in applicants' laboratory and determined to yield exactly the same amount of hybridomas when compared to the parental cell line X63-Ag8.653 (158 hybridomas per mouse vs 155 hybridomas).

Applicants hereby submit the data of the experiment resulting from this testing in applicants' laboratory.

Empirical Data

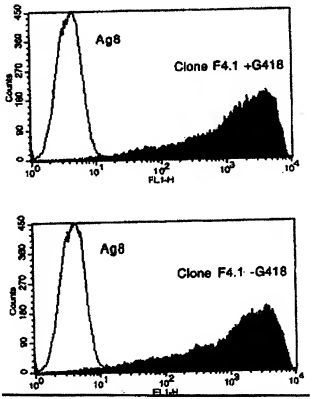
In the experiment, conventional hybridoma technology was used – either myeloma cell line X63-Ag8.653 or its protein G displaying derivative (see below) were fused to B-lymphocytes from an immunized mouse donor to generate hybridoma cells.

The main purpose of the experiment was to rule out negative side effects of protein G-binding antibodies on the growth of resulting hybridomas, especially on their shared export pathway through the endoplasmic reticulum. No such negative side effect was found, and it is respectfully submitted that anyone skilled in the art of generating

hybridoma cells should as a matter of such ordinary skill in the art be able to generate hybridomas with the "starting material" disclosed by applicants.

The fused hybridoma cells initially have both the antibody binding protein (from the myeloma partner) together with the antibody (from the B-lymphocyte).

Immediately after fusion to hybridoma cells the "random shedding of genetic elements from hybridomas" (Office's statement at page 3, line 20) results in the generation of cells without surface displayed antibodies (either due to lost antibody genes or lost antibody binding protein) and such "surface antibody-less" cells therefore are present together with cells that still display antibodies on their surface. Applicants respectfully submit that the selection of cells, and certainly of hybridoma cells, based on surface displayed proteins, such as by means of a fluorescent label in a FACS sorter, or by means of magnetobeads covered with a suitable binding protein or antigen, is within the knowledge and skill of the state of the art, when such knowledge and skill are informed by applicants' present disclosure. The Office's statement that there is "... the need to select relevant cells secreting relevant antibodies from the population of fused cells" is consistent with the notion that a method enabling the parallel screening of a mixture of hybridoma cells for surface displayed antibodies of a desired kind, constitutes a substantive contribution to the existing state of the art.



The Office, however, disputes that a "large amount" of antibody binding proteins needed for surface displayed antibodies is provided by applicants' invention, and the Office contends that "this is not supported by any disclosure or evidence of record."

Applicants in response respectfully direct the Office's attention to the fact that the disputed "large amount" of antibody binding proteins is indeed disclosed, namely as an intrinsic property of the disclosed myeloma cell line derived from X63-AG8.653 which was transfected with the plasmid pSEX15G2 (see Fig. 3), and then selected for a strong antibody-binding signal.

To substantiate such contention, applicants attach hereto (in Appendix A hereof)[1] FACS data that characterize the applicant's stable myeloma cell line (descendent of myeloma cell line X63-Ag8.653 that expresses two protein G binding domains displayed on the surface and generated by transfection with pSEX15G2 that is described in Fig 3).

The protein G-domain-expressing cell line was grown in RPMI medium + 10% FCS with or without continued selective G418 addition. It is to be noted that the staining (FITC labeled goat anti cow antibodies) resulted into a very strong signal indeed (up to the fourth decade), irrespective of continued selective pressure (G418), thereby substantiating applicants' claim of a "large amount" of antibody binding proteins.

Furthermore, it is undisputed state of the art that at least directly after fusion in a resulting hybridoma cell both the membrane-bound antibody binding protein and newly made antibody proteins together travel down the endoplasmic reticulum (ER), and such fact

[1] See enclosed figures and appertaining histogram statistics in Appendix A. Hybridoma cells were generated by conventional hybridoma technology from B-lymphocytes derived from an immunized mouse donor (BALB/c). B-lymphocytes were fused either to myeloma cell line X63-Ag8.653, or to its derivative stable cell line originally transfected with pSEX15G2 (Example 1B). The resulting hybridoma cells were selected in HAT medium and grown in RPMI medium + 10% FCS with new medium added every 48 hours. Individual antigen specific cell clones were stained with FITC-labeled goat-anti-mouse-IgG antibodies and analyzed 14 days post fusion by FACS.

indicates that sufficient quantities of antibody binding protein and newly made antibodies meet each other in the ER.

Applicants further point to the fact that numerous publications, including textbooks (e.g., Goding, J. W. "Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology. Third Edition, 1996, Academic Press London) describe a strong affinity of protein G towards antibody Fc-domains (for protein L, see Akerstrom B and Bjorck BH, 1989, J. Biol. Chem. 264, 19740-19746). Other publications describe the binding of antibody fragments towards their antigen within the ER, e.g., Marasco et al. (1993, PNAS 90, 7889-7893).

Applicants therefore point out the following:

- that applicants have generated a stable and easy-to-handle cell line that behaves exactly like the parental myeloma cell line X63-Ag8.653 with respect to the yield of hybridoma cells,
- that the generation of hybridoma cells has for the last 30 years been a widespread method, with hardly any other methods being better documented state of the art,
- that the present invention involves the generation of hybridoma cells employing state of the art hybridoma technology, in which the myeloma fusion partner is replaced by a myeloma cell that expresses a large amount of antibody binding protein,
- that this modified myeloma cell and the DNA used to generate it, respectively, are indeed disclosed and available to the scientific community,
- that the myeloma cell's expression of a "large amount" of antibody binding protein is evidenced by data included herewith,
- that it is well known state of the art that both antibodies and antibody binding proteins travel the same export pathway down the ER,
- that antibody Fc-moieties are high-affinity binders for the applicants' example of an antibody binding protein, namely protein G, and

- that there are many publications that describe a binding of an antibody fragment to an antigen within the ER.

Given this wealth of evidence, applicants therefore respectfully dispute the Office's notion that applicant's claim "is not supported by any disclosure or evidence of record."

Moreover, given the facts shown above, applicants strongly dispute the Office's statement that "one skilled in the art ... would not be assured of the ability to select the desired producer cell(s) from the population because, absent further guidance from applicant, one would be unable to identify and specifically separate the secreting cell(s) from a population of cells which are capable of binding the secreted product."

In further support for applicants' position, applicants attach FACS data that evidence surface displayed antibodies of hybridoma cells stained by anti-mouse antibodies, even when grown in a huge excess of competing antibodies derived from fetal calf serum (FCS).

Hybridoma clones derived from the fusion with the protein G domain engineered myeloma cell line described above, were grown in RPMI medium plus 10% FCS and then stained with FITC-labelled-anti-mouse-IgG.

If there were no "kinetic advantage" due to pre-loaded antibody-protein G complexes within the ER, there should be virtually no binding signal due to competition of excess calf antibodies. It is to be noted that calf antibodies bind especially tightly to protein G, and that 10% FCS translates to roughly 1 – 2mg /ml cow IgG antibodies.

The results show that some hybridomas do not bind anti-mouse-IgGs, presumably due to reduced levels or complete loss of antigen binding protein. Moreover, most clones display a majority of cells with only a small signal due to surface displayed antibodies. That this could happen was correctly described by the Office in the sentence "what other properties

are retained by such fused cells would seem to be entirely unpredictable because of the ... random shedding of genetic elements from hybridomas" (page 3, lines 18-20 of the February 16, 2006 Office Action).

However, almost invariably a minority of cells within individual clones of hybridoma cells – although initially screened by conventional hybridoma technology – still binds 14 days post fusion to anti-mouse-IgGs, which is only explainable by the kinetic advantage mentioned above, though disputed by the Office. Applicants respectfully point out that this signal is strong enough to allow, for example, for FACS sorting of hybridoma cells due to surface displayed antibodies, within the knowledge and skill of the state of the art, when such knowledge and skill are informed by the disclosure of the present application.

Applicants also point to the fact that this staining was done under conditions strongly disfavoring binding of antibodies produced by the hybridoma cell to its own surface, i.e., conditions involving:

- an excess of competing cow antibodies (10% FCS), and
- repeated cultivation of hybridomas in new medium in order to prove that there is only a small influence of cross talk between cells in this experimental setting, i.e., cross-talking antibodies are nearly 100% cow antibodies while displaced antibodies are nearly 100% mouse antibodies.

Even under these artificially disadvantageous staining conditions, the majority of hybridoma cells give a clear FACS signal when stained with FITC-labelled-anti-mouse-IgG, which is explainable only with the kinetic advantage described above.

Further, applicants point to the obvious fact that it is easy to design experimental conditions more favorable for surface display, e.g., omitting FCS, and it is easy to "select relevant cells secreting relevant antibodies from the population of fused cells" in accordance with the applicants' invention (see in this respect the preceding discussion

herein responding to the Office's statement that there is "... the need to select relevant cells secreting relevant antibodies from the population of fused cells").

Thus, the disclosure of the invention in the present application enables one of ordinary skill in the art to do FACS sorting employing a pool of hybridoma cells every two or three days in order to sort out those cells that retain displayed antibodies and thereby to avoid laborious cell cultivation of single colonies until the "entirely unpredictable ... random shedding of genetic elements from hybridomas" has come to an end.

CONCLUSION

Based on all of the foregoing, it is requested that the examiner take cognizance of the remarks and evidence presented herein, and (i) confirm the acceptability of the drawing sheets for FIG. 3B submitted in the application on September 20, 2005 as being in compliance with the requirements of 37 CFR 1.84, and (ii) withdraw the objection to the specification and the rejection of claims 1-14 and 21-24 on 35 USC 112, first paragraph grounds.

Respectfully submitted,

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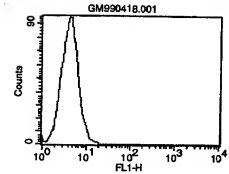
APPENDIX A



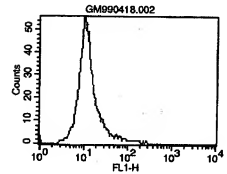
Ag8 -Kontr. GAM-FITC

A8

Histogram Statistics

File: GM990418.001
Sample ID: Ag8 -Kontr. G
Acquisition Date: 18-Apr-99
Gated Events: 10000
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	4.46	4.07



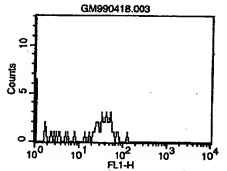
Ag8 Klone N GAM-FITC

N4

Histogram Statistics

File: GM990418.002
Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99
Gated Events: 5054
Total Events: 5054

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	5054	100.00	16.66	11.14



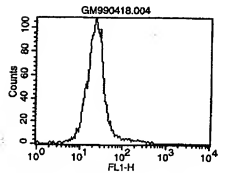
Ag8 Klone N GAM-FITC

N10

Histogram Statistics

File: GM990418.003
Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99
Gated Events: 107
Total Events: 107

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	107	100.00	30.41	33.08



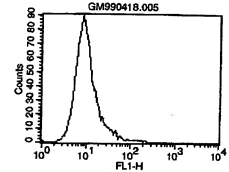
Ag8 Klone N GAM-FITC

N15

Histogram Statistics

File: GM990418.004
Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99
Gated Events: 10000
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	25.37	21.67



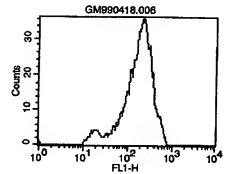
Ag8 Klone N GAM-FITC

18

Histogram Statistics

File: GM990418.005
Acquisition Date: 18-Apr-99
Total Events: 10000
Sample ID: Ag8 Klone N G
Gated Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	12.28	8.90



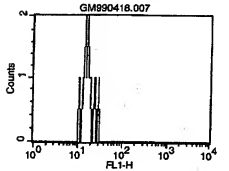
Ag8 Klone N GAM-FITC

23

Histogram Statistics

File: GM990418.006
Acquisition Date: 18-Apr-99
Total Events: 5053
Sample ID: Ag8 Klone N G
Gated Events: 5053

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	5053	100.00	197.45	181.06



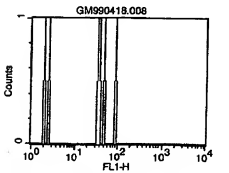
Ag8 Klone N GAM-FITC

24

Histogram Statistics

File: GM990418.007
Acquisition Date: 18-Apr-99
Total Events: 20
Sample ID: Ag8 Klone N G
Gated Events: 20

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	20	100.00	16.74	15.40



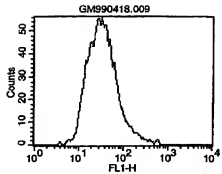
Ag8 Klone N GAM-FITC

29

Histogram Statistics

File: GM990418.008
Acquisition Date: 18-Apr-99
Total Events: 7
Sample ID: Ag8 Klone N G
Gated Events: 7

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	7	100.00	33.14	33.98



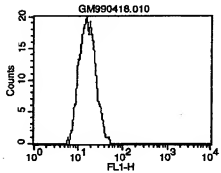
Ag8 Klone N GAM-FITC

33

Histogram Statistics

File: GM990418.009 Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99 Gated Events: 10000
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	43.25	30.78



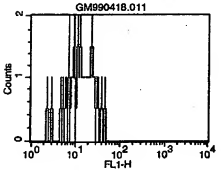
Ag8 Klone N GAM-FITC

44

Histogram Statistics

File: GM990418.010 Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99 Gated Events: 2048
Total Events: 2048

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	2048	100.00	17.08	15.54



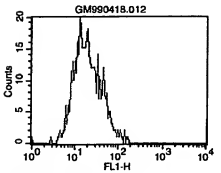
Ag8 Klone N GAM-FITC

54

Histogram Statistics

File: GM990418.011 Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99 Gated Events: 51
Total Events: 51

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	51	100.00	15.61	13.34



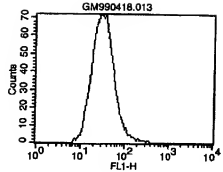
Ag8 Klone N GAM-FITC

55

Histogram Statistics

File: GM990418.012 Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99 Gated Events: 2057
Total Events: 2057

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	2057	100.00	22.27	17.00



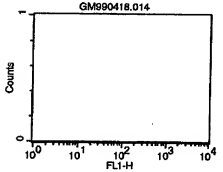
Ag8 Klone N GAM-FITC

56

Histogram Statistics

File: GM990418.013
Acquisition Date: 18-Apr-99
Total Events: 10000
Sample ID: Ag8 Klone N G
Gated Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	38.00	29.43



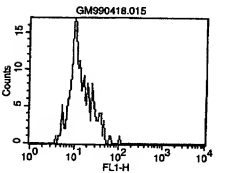
Ag8 Klone N GAM-FITC

63

Histogram Statistics

File: GM990418.014
Acquisition Date: 18-Apr-99
Total Events: 24
Sample ID: Ag8 Klone N G
Gated Events: 24

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	24	100.00	33.13	32.63



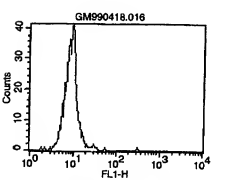
Ag8 Klone N GAM-FITC

64

Histogram Statistics

File: GM990418.015
Acquisition Date: 18-Apr-99
Total Events: 1020
Sample ID: Ag8 Klone N G
Gated Events: 1020

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	1020	100.00	14.96	11.97



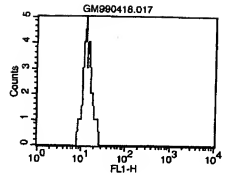
Ag8 Klone N GAM-FITC

72

Histogram Statistics

File: GM990418.016
Acquisition Date: 18-Apr-99
Total Events: 2077
Sample ID: Ag8 Klone N G
Gated Events: 2077

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	2077	100.00	8.72	8.20



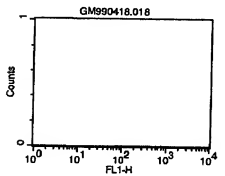
Ag8 Klone N GAM-FITC

73

Histogram Statistics

File: GM990418.017
Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99
Gated Events: 236
Total Events: 236

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	236	100.00	15.77	13.10



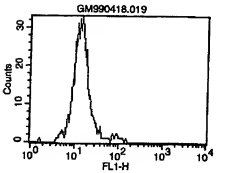
Ag8 Klone N GAM-FITC

75

Histogram Statistics

File: GM990418.018
Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99
Gated Events: 4
Total Events: 4

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	4	100.00	29.76	36.52



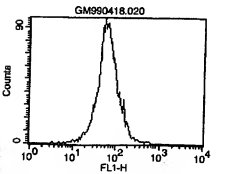
Ag8 Klone N GAM-FITC

93

Histogram Statistics

File: GM990418.019
Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99
Gated Events: 2366
Total Events: 2366

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	2366	100.00	15.40	13.34



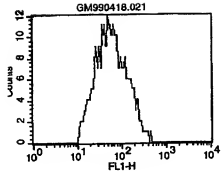
Ag8 Klone N GAM-FITC

100

Histogram Statistics

File: GM990418.020
Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99
Gated Events: 10000
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	68.56	58.82



Ag8 Klone N GAM-FITC

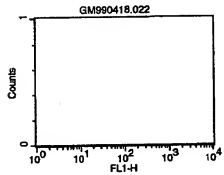
101

Histogram Statistics

File: GM990418.021
Acquisition Date: 18-Apr-99
Total Events: 2053

Sample ID: Ag8 Klone N G
Gated Events: 2053

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	2053	100.00	73.29	53.76



Ag8 Klone N GAM-FITC

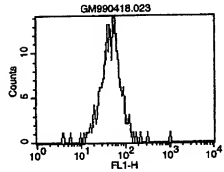
104

Histogram Statistics

File: GM990418.022
Acquisition Date: 18-Apr-99
Total Events: 3

Sample ID: Ag8 Klone N G
Gated Events: 3

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	3	100.00	96.24	85.05



Ag8 Klone N GAM-FITC

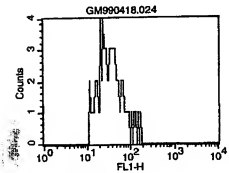
113

Histogram Statistics

File: GM990418.023
Acquisition Date: 18-Apr-99
Total Events: 1015

Sample ID: Ag8 Klone N G
Gated Events: 1015

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	1015	100.00	51.11	45.73



Ag8 Klone N GAM-FITC

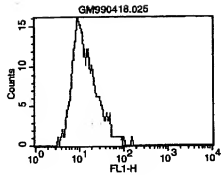
113

Histogram Statistics

File: GM990418.024
Acquisition Date: 18-Apr-99
Total Events: 206

Sample ID: Ag8 Klone N G
Gated Events: 206

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	206	100.00	36.93	31.62



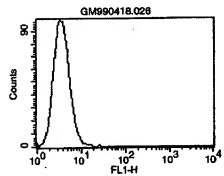
Ag8 Klone N GAM-FITC

A20

Histogram Statistics

File: GM990418.025 Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99 Gated Events: 2035
Total Events: 2035

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	2035	100.00	17.50	12.30



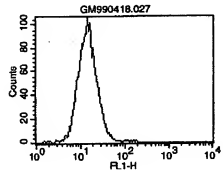
Ag8 Klone N GAM-FITC

A21

Histogram Statistics

File: GM990418.026 Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99 Gated Events: 10000
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	3.89	3.55



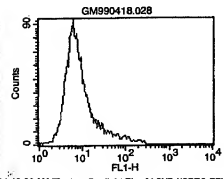
Ag8 Klone N GAM-FITC

A31

Histogram Statistics

File: GM990418.027 Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99 Gated Events: 10000
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	15.77	14.07



Ag8 Klone N GAM-FITC

A41

Histogram Statistics

File: GM990418.028 Sample ID: Ag8 Klone N G
Acquisition Date: 18-Apr-99 Gated Events: 10000
Total Events: 10000

Marker	Left, Right	Events	% Total	Mean	Median
All	1, 9910	10000	100.00	12.20	6.98